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Colonization of newly fallen leaves by micro-organisms

INTRODUCTION

Newly fallen leaves are little attractive for most litter-eating animals. If, however, the leaves have for some time been lying on the ground they are readily eaten. It would seem that a certain concentration of bacteria and fungi has to develop in the litter to make it palatable. The microflora that develops has to be considered as the most important source of nutrition (mainly easily digestable protein) for the litter-eaters, whereas the leaf-tissue itself mainly plays the rôle of a substratum that has to be taken up in order to obtain the real nutrition. Chemical and microscopical composition of faeces strongly support this hypothesis.

The degree of colonization of foliage by micro-organisms can be studied by means of plate counts. However, one may expect that this method does not give much insight into the real density of the micro-organisms. The material is not very suitable for making the well dispersed suspensions which are required for dilution-series.

Research on nematodes (M i n d e r m a n 1956) has provided a technique for direct observation of the microflora. Brown foliage is first bleached and then stained with a protoplasm stain, after which the organisms are sufficiently contrasted against their background. The total number of bacteria that are present, and the length of mycelium can be determined with reasonable accuracy.

METHOD

Periodically litter-samples were collected from the same layer, in the present case the undermost layer of newly fallen foliage. After fixation in 4% formaldehyde for 24 hours the samples were bleached in 6% H_2O_2 to which some ammonia had been added. After the brown leaf-colour had changed to a pale creamy colour (sometimes with green spots, because colonies of algae became visible), the bleaching process was terminated by putting the leaves in water.

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The bleached leaves can be stained, e.g., by chlorazol-black in alcohol, or, as we mostly did, by chlorazol-black in lactophenol. In the latter case we stained for 5 - 10 minutes at temperatures between 65 - 70° C. We then washed in lactophenol till no dye came off.

A number of discs of a diameter of about 5 mm were punched out of the leaves. 10 - 20 of such discs were embedded in lactophenol under a large coverslip on a slide, and the mount sealed with "glyceel".

After a preliminary study of the organisms with an oil-immersion objective, we could count them by means of an apochromatic objective 25 x, and an eye-piece 12.5 x. We chose a Zeiss Integration eye-piece I, which proved to be particularly suitable for counting.

In each sample we counted the organisms in 40 fields of vision under the microscope, one field of vision being 0.11 mm^2. By determining the average cross-section of a bacterium it was possible to calculate that 325.000 bacteria would fill the field of vision. By determining the number of times that in a certain field of vision bacteria coincide with ("hit") intersections in the counting-grid one can estimate the number of bacteria in the field of vision. One "hit" was equal to 2.8×10^5 bacteria per cm^2 of leaf.

Mycelium length was determined by multiplying each intersection of hyphae and grid-line with the distance between two grid-lines as projected on the field of vision.

The counts were first expressed per cm^2 of leaf, and then per gram of dry leaf (the average weight of 1 cm^2 of dry leaf was 7.9 mg).

Hering and Nicholson (1964) have published a different staining technique, which according to them gave better results. They developed their method after having had difficulties with our method of staining. We did not apply their technique, for the present paper is based on work that was carried out in 1959 and 1960.

RESULTS

The observations were made from October 9, 1959 till June 2, 1960 in our experimental area Hackfort. This is a rather young oak-forest on an area, which for centuries has been a forest. The soil composition varies from loamy sand to very coarse sand, with locally $CaCO_3$ in the topsoil. The herbaceous undergrowth is very varied, from poor to very

rich. The soil water regime shows rather great variations over short distances. The types of forest floors in relation to the soil water regime have been described by Minderman (1960).

The summer and autumn of 1959 were exceptionally dry. The bone-dry brown leaves fell on a desiccated forest-soil. The rains began on October 19, and on October 30 the top-soil was somewhat moistened to a depth of about 8 cm. At a lower level the soil was still dry. On October 9 the first sample was taken. This consisted of leaves that had fallen dry from the trees and that had lain dry on the ground. The sample was crisp dry. It contained only bacteria, all concentrated on the outer surface. On October 30 regular sampling began, at first weekly on each of the three types of forest floor present, viz., I. mull rich in lime, II. acid mull, and III. mor (Minderman 1960).

The counts of bacteria and fungi are summarized in three graphs (Fig. 1). The data show that the microbe population reached a high level soon after the leaves were moistened.

The highest numbers of bacteria were found in the first weeks. They soon decreased to a level that from February till June remained constant.

The fungi in the middle of November rather suddenly appeared to be present in considerable quantity. Those in forest floor type I and II reached a constant level near the turn of the year, but those in type III were at that time still increasing considerably.

All mycelia with a thickness of about 1 μ were considered to be Actinomycetes, and these turned up all at once in great quantity, a week after the fungi. They immediately reached a level that was maintained during the period of observation.

Our observations give no information on the speed of succession of generations and species "turn-over": they are but static snapshots.

DISCUSSION

The numbers of bacteria are high. We found 2 - 22 x 10^9 per gram of dry organic matter. These figures should be compared with those obtained from plate-counts that relate to soil samples and to litter more than one year old (the L_2-layer), figures that were calculated per gram of organic matter (Went 1966).

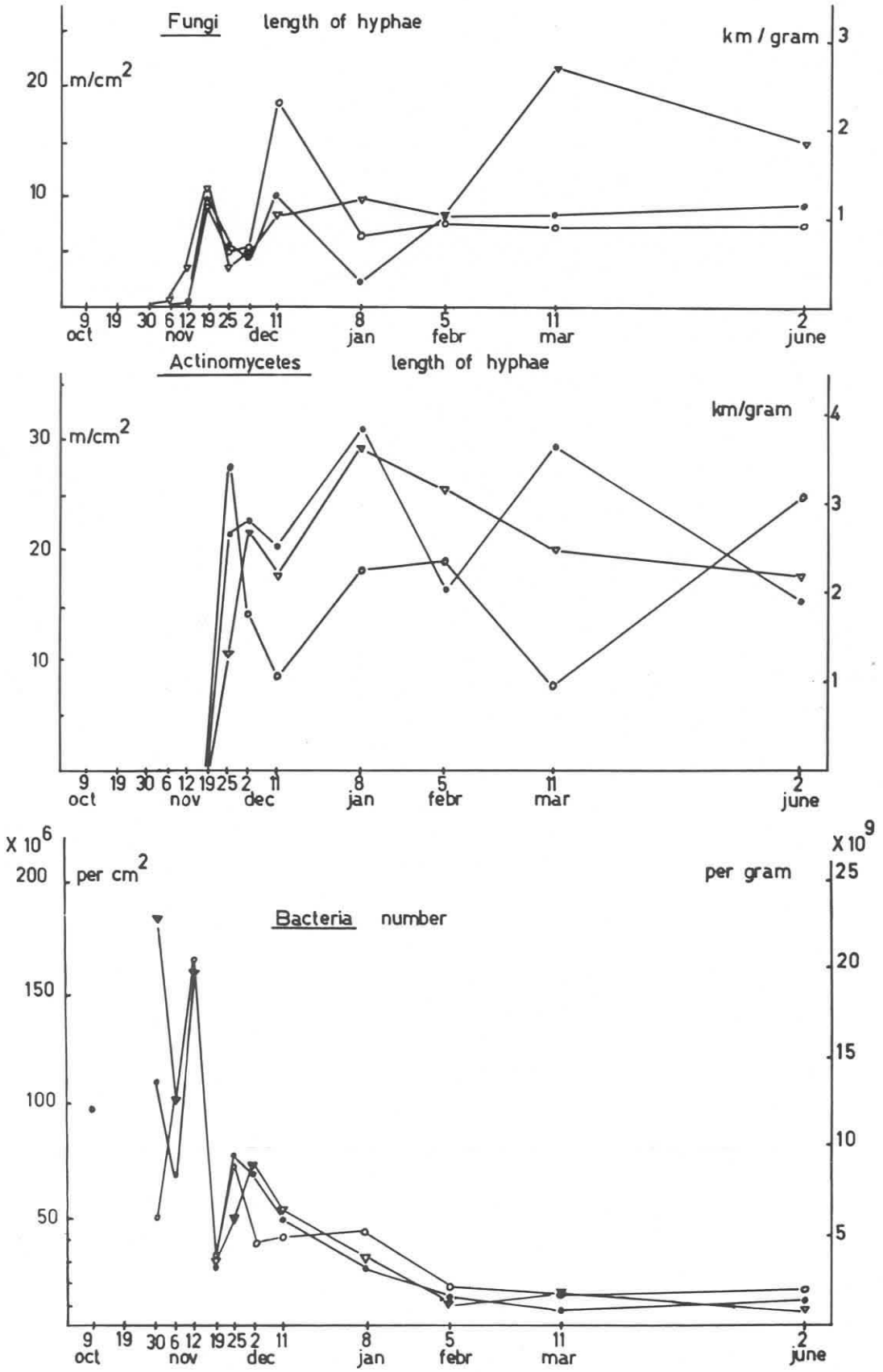

Fig. 1

Colonization of oak-forest-litter, from the falling of the leaves till the following summer Number of bacteria and total length of mycelium per cm^2 leaf surface and per gram of organic dry matter. o mull rich in lime, ● acid mull, Δ mor

	Type I	Type II	Type III
L_2-layer	$0.6 - 2 \times 10^9$	$0.2 - 1 \times 10^9$	$0.03 - 0.5 \times 10^9$
At a depth of 5 cm	0.4×10^9	0.2×10^9	0.02×10^9
At a depth of 10 cm	0.3×10^9	0.02×10^9	0.01×10^9

As to the quantity of mycelium,a comparison can be made with data obtained by Nagel-de Boois and Jansen (1966). They found in the toplayer of the mineral soil in:

Forest floor type I 1800 m of mycelium/gram organic matter
" " II 1800 " " "
" " III 4600 " " "

However, their data on mineral soil cannot without further consideration be compared with what we found in litter. For it appears that with increasing depth the length of mycelium per gram of organic matter shows a very considerable increase. This cannot be ascribed to a greater amount of assimilable organic matter at greater depth, on the contrary (Minderman 1966). It is caused by a phenomenon recorded by Nagel-de Boois. She found that with increasing depth the average "life-span" of mycelium increases. This is a result of less activity of mycolytic bacteria in the deeper layers of the soil. It is unknown, however, what the activity is of these mycolytic bacteria in litter.

LITERATURE

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DISCUSSION

C.A. EDWARDS: I found Dr. Minderman's lecture on the colonization of litter by fungi and bacteria most interesting. However, I should like to know the justification for the statement in his introduction that a certain concentration of bacteria and fungi have to develop in the litter to make it palatable. I agree that after leaves have lain on the ground for some time they are more readily eaten. They are, however, also eaten by the fauna before this.
We have found that if leaves are artificially leached for some time they become more palatable, and we have attributed this to loss of watersoluble polyphenols. I agree that we cannot be certain which substances are leached, because a lot of other substances will also go into solution. But it seems to me the time factor is only part of the answer for there are changes in microflora due to leaching. Our work has also indicated that microorganisms follow after fragmentation by microarthropods in the early stages of breakdown.

G. MINDERMAN: As far as I know freshly fallen leaves will as a rule not be eaten, exept in culture dishes if only these fresh leaves are offered. From our work, fragmentation was not a necessary precedent for the growth of microorganisms. We used whole leaves or large pieces.
Very soon after the thorough wetting of the leaves in the ground layer there is the development of a high population of bacteria and fungi. Then the leaves become densily populated by arthropods, lumbricides and nematodes. As a rule, the latter show in their faeces a considerable portion of fragmented hyphae besides leaf debris. In general, the nematodes feed exclusively on hyphae contents or bacteria. The idea is that proteins out of bacteria and fungi are much easier to use by the fauna than the residual leaf proteins, mostly bound to tannin substances.
Up to now I have no clear-cut idea concerning the substances that will leach out in the first step of litter breakdown. A large amount of "tannin-condensates" will stay behind and will resist leaching. However, the thorough wetting of the leaves, or the artificial leaching of water soluble substances, is of importance as to the palatability.

G. JAGNOW: Do your figures apply to upper- and under-side of leaves, that means per cm^2 upper and cm^2 under side = per cm^2 leaf area?

G. MINDERMAN: The figures apply to the total number of organisms present in the three dimensional space of a leaf between upper and under epidermis under each square cm.

S.A. VISSER: I have always thought that fungi, and to a minor extent also the actinomycetes, were to be regarded as the first colonizers in respect of the breakdown of fresh organic matter (which usually has a low pH). Your results, however, do not seem to bear this out. Could you please comment on this?

G. MINDERMAN: Our findings are as they appeared to us and are in accordance with those of others. (Please refer to the remarks of Szabó and Heal of this paper.)

W. BANAGE: In relation to the view that newly fallen litter is not readily eaten and has to be first conditioned by bacteria, has any experiment been tried using unacceptable newly-fallen litter, inoculating it artificially with higher bacterial populations to see if then it becomes more acceptable, before it has started to decompose?

G. MINDERMAN: I refer to the comments of Dr. Edwards to this question, with which I wholly agree.

C.A. EDWARDS: I should like to ask two questions. First, have you examined any other site for comparison? Second, following Dr. Minderman's question, I am interested in how you assess the absolute efficiency of your sampling methods We are working with many groups of animals in this way and find such assessment very difficult even with very large animals.

I. SZABO: Up till now, there has been little data about the occurance and population-dynamics of actinomycetes in litter. This investigation shows that the change in the total number of these organisms follows the cycle of microbiological activity so common in soils. That is, the first stage of decomposition is done by bacteria growing on the easily utilizable compounds. Later the actinomycetes and fungi grow saprophytically on the disintegrated protoplasmic elements of the bacteria.

O.W. HEAL: Using the Jones & Mollison (1948) direct counting method at Merlewood, we have found similar numbers of bacteria and fungi on oak litter. Mr. Nicholson at Merlewood also found a rapid increase, then fall in number of bacteria followed by a slower increase in the quantity of fungi on the faecal pellets of *Glomeris*.

Converting Dr. Minderman's numbers of microorganisms per gram of leaf to biomass suggests that less than 1% of the dry matter is microbial. This would not be enough to satisfy the demands of litter-feeding animals.

Can you relate the changes in the microflora populations to decomposition of the organic matter? The knowledge of the chemistry for this is still too incomplete to pass the border of supposition.